Environ. Sci. Technol. 1996, 30, 422–430

Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test

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The physiologically based extraction test (PBET) is an *in vitro* test system for predicting the bioavailability of metals from a solid matrix and incorporates gastrointestinal tract parameters representative of a human (including stomach and small intestinal pH and chemistry, soil-to-solution ratio, stomach mixing, and stomach emptying rates). For lead (Pb), the results of the PBET are linearly correlated with results from a Sprague-Dawley rat model ($r^2 = 0.93$ between *in vitro* and *in vivo* results, $n = 7$). For arsenic (As), the results of the PBET are overpredicting bioavailability study results in rabbit and primate models (2–11% difference between *in vitro* and *in vivo* results, depending on the animal model). The PBET was not designed to supplant bioavailability studies using animal models, but rather to estimate Pb and As bioavailability when animal study results are not available. Dissolution of Pb in the acidic stomach environment was strongly pH dependent; the extent of dissolution decreased by 65% when stomach pH was increased from 1.3 to 2.5. Arsenic solubility decreased by only 16% over the same pH range. Lead was removed from solution to a greater extent than As by neutralization during the small intestinal simulation, consistent with adsorption and precipitation reactions occurring for Pb—but not As—at neutral pH values. In addition to providing mechanistic explanations for controls on Pb and As bioavailability, the PBET allows estimates of site-specific Pb and As bioavailability from soil for the purpose of exposure assessment.

Introduction

When assessing risks associated with lead (Pb)-contaminated soils, one exposure pathway typically evaluated is soil ingestion by children. Standard procedures recommended by the U.S. Environmental Protection Agency (EPA) for estimating soil Pb exposures in children assume that 30% of ingested soil Pb will be absorbed into the systemic circulation (i.e., will be bioavailable) (*1*). However, recent studies suggest that Pb bioavailability may be dependent on the form and solubility of Pb present (*2–4*) and site-specific soil chemistry (*5*). Studies in rats of the bioavailability of Pb derived from mining wastes or smelter emissions have confirmed the dependence of Pb bioavailability on Pb form (*6–9*). Therefore, application of a default 30% bioavailability value may not be appropriate for all types of Pb contamination.

To address this issue, development and validation of a physiologically based extraction test (PBET) for site-specific estimation of soil Pb bioavailability was undertaken (*10*). This test was based on the premise that the form and solubility of Pb in a soil or mine waste will control its bioavailability in an animal model or in humans. This paper describes method development and validation of an *in vitro* test method that is predictive of Pb bioavailability in an animal model. In addition, the PBET has been used to screen for Pb bioavailability of Pb-containing soils and waste materials to determine whether a Pb bioavailability study in an animal model would be warranted. The PBET also appears to be useful in studying the gastrointestinal (GI) tract parameters that control lead bioavailability (e.g., stomach pH and residence time, lead mineralogy, and soil type) and in evaluating the efficacy of *in situ* soil amendments designed to reduce lead bioavailability.

In evaluating arsenic (As)-related risk, the U.S. Environmental Protection Agency (EPA) has derived a cancer slop factor (CSF) and a reference dose (RfD) for use in assessing the cancer risks and other noncancer adverse health effects, respectively, that might be associated with oral exposures to As (*11*). Oral toxicity values typically are derived from animal or human studies that characterize adverse health effects in response to an orally administered dose. However, the administered dose of a compound is seldom completely absorbed, and for many compounds, there are significant differences in the extent of oral absorption from different media. This can lead to overly conservative and costly assumptions in assessing the potential risk of exposure to a particular compound in a medium other than the one used in the studies on which toxicity values are based. The oral toxicity values for As were derived from epidemiological studies of As in drinking water (*12, 13*). Studies investigating the absorption of soluble As ingested by humans suggest that close to 100% of soluble inorganic As is absorbed from the gastrointestinal tract (*14*). In contrast to As in drinking water (soluble As), As in soils generally exists as mineral forms or soil–As complexes that will be incompletely solubilized during transit through the gastrointestinal tract. Recent research indicates that As must be dissolved in order to be absorbed (*15*); therefore, As in soil will be less well absorbed than arsenic in drinking water. Therefore, a bioavailability adjustment factor may be necessary to accurately assess potential risks associated with ingestion of soil As by correcting for the difference in absorption between As in soil and in drinking water.

Reduced absorption of arsenic from soils, relative to

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0013-936X/96/0930-0422$12.00/0

soluble forms, has been demonstrated in rabbits, dogs, microswine, and monkeys. Arsenic bioavailability from Netherlands soils produced only 8.3% absolute As bioavailability in dogs, when compared to intravenous administration of soluble As (*16*). The soil tested in this study was described as bog ore, with arsenic bound to clay surfaces within it, specifically iron and aluminum oxides. Soil samples affected by smelter emissions were used in studies of New Zealand White rabbits and *Cynomolgus* monkeys, resulting in 48 and 20% As bioavailability, respectively, relative to soluble As (*17*, *15*), based on urinary As data. House dust studied in the *Cynomolgus* monkey model resulted in 28% relative As bioavailability (*15*). Recent studies of As bioavailability from mining and smelting wastes in a microswine model also indicate reduced As bioavailability relative to soluble As. These studies indicate that As adsorbed to organic material or clay surfaces in soils, or present as As mineral phases, results in limited As bioavailability in animal models. Because As bioavailability appears to be limited by As form and solubility, the PBET was also developed for As bioavailability estimation. The PBET provides a tool for screening site-specific exposure to As in the absence of As bioavailability data from an animal model. The bioavailability adjustment determined from the PBET can be incorporated into an As risk assessment by use of a bioavailability adjustment factor (ranging from 0 to 1) to adjust soil As exposure estimates so that they are expressed in the same terms as water As exposure estimates.

For the purpose of this paper, the absolute bioavailability of Pb and As is defined as that fraction of ingested Pb and As that is absorbed into systemic circulation. The term "relative bioavailability" is used to describe the bioavailability of Pb and As in mine waste or soil relative to that of Pb and As dissolved in water. Because the bioavailability of Pb and As dissolved in water has been determined in humans, the measurement of relative bioavailability in animal models allows correction of these experimental values back to humans. For example, the bioavailability of Pb in the diet of children (e.g., soluble Pb) is approximately 50% (*1*). If relative Pb bioavailability from a particular soil was determined to be 20% in an appropriate animal model, then this value could be corrected for the known uptake of soluble Pb in children (correction factor of 0.5) to arrive at an estimated 10% absolute Pb bioavailability for this substrate in children. This extrapolation from the animal model to children does not require that the animal mimic or be equivalent to children, but rather that Pb from the test material behave proportionally the same relative to soluble Pb in children and the animal model. Similarly, because the bioavailability of As dissolved in water has been determined in humans, the measurement of relative As bioavailability in animal models provides the most relevant data for correction back to a human model. Finally, the term "bioaccessibility" is used to define the fraction of total Pb and As that dissolves in the stomach and is available for absorption during transit through the small intestine. The bioaccessibility of Pb and As provides a measure of solubility in the gastrointestinal tract. The fraction of bioavailable Pb and As will be less than the fraction of bioaccessible Pb and As, due to incomplete uptake of solubilized Pb and As in the small intestine.

The PBET was designed around pediatric GI tract parameters for a child 2–3 years old, believed to be at the greatest risk to metal exposure from accidental soil ingestion. This approach was taken to develop a test based on the biological model of interest (e.g., humans, specifically, children) based on the premise that replicating the conditions in the model of interest would produce the most relevant data. Because bioavailability of the test materials could not be measured directly in children, the PBET could not be validated against this particular model. Therefore, animal models were relied upon for comparison to the PBET results, based on the premise that data from appropriate animal models can be extrapolated to humans for the purpose of exposure assessment. Thus, the validation of the PBET is specific to the animal models used, and the usefulness of the test in predicting human exposure is based on extrapolation of these animal data to humans.

It should be noted that the PBET is a screening-level test and does not mimic the entire physiological process controlling uptake of Pb and As. It has not been designed to simulate transport of Pb and As across the intestinal epithelium. As a result, the test cannot evaluate the dose dependency of absorption. *In vivo* studies indicate that saturation of uptake mechanisms results in dose-dependent uptake of Pb (*6*, *18*, *19*), causing uptake to decrease at higher Pb doses; this phenomenon has not been observed for As at environmental dose levels (*17*). Because the PBET tests only for Pb solubility constraints, it will tend to overestimate Pb bioavailability if dose-dependent uptake causes a significant reduction in Pb uptake. Pb and As absorption in the small intestine may also create a disequilibrium in the small intestinal fluid, which results in additional dissolution of Pb and As (i.e., the small intestine provides a sink for Pb and As); this eventuality has not been evaluated in the PBET. In addition, the PBET, as described herein, does not account for the presence of food in the gastrointestinal tract. Although nutritional status is known to effect Pb and As uptake (*20*), the PBET has been used to mimic fasting conditions, which produce the most soluble Pb and As, and, hence, the most conservative condition. Despite these limitations, which are the topic of ongoing research, the PBET appears to produce data that correlate well with measures of Pb and As bioavailability in animal models.

This paper provides data for seven Pb and three As substrates that were evaluated using both the PBET and animal models, compares the PBET results to those from the animal bioavailability studies, and suggests a screening-level method for extrapolating PBET data to evaluations of human exposure to Pb and As in soil.

Test Materials

Substrates evaluated for Pb bioaccessibility in the PBET included two composite mine waste materials from Butte, MT (BMW-I and BMW-II, Table 1); two composite residential soil samples from the vicinity of historical zinc and lead smelters in Bartlesville, OK (BVS) and the Salt Lake City, UT (SCS) areas, respectively; two composite tailings samples from the Copperton tailings, UT (CT-1 and CT-2); and a composite stream channel sample from the Bingham Creek channel, UT (CT-3). The Bingham Creek channel sample contains Pb that is probably related to historical mining and milling operations conducted in the area. These substrates were tested for Pb bioavailability using dosed-feed oral bioavailability studies in Sprague-Dawley rats (*6*, *8*, *21*, *22*), allowing comparison of the PBET results to bioavailability data from a rat model.

Arsenic bioaccessibility was evaluated in two composite residential soil samples (ARS-I and ARS-II, Table 2) and

TABLE 1

Lead Test Material Composition

sample origin	Butte mine waste no. 1	Butte mine waste no. 2	Bartlesville soil	Salt Lake City soil	Copperton tailings no. 1	Copperton tailings no. 2	Bingham Creek Channel
sample identifier	BMW-I	BMW-II	BVS	SCS	CT-1	CT-2	CT-3
lead concn (mg/kg)	3940	3908	1388	2090	7220	6890	10 230
pH	3.6	3.7	7.0	7.5	2.4	2.8	4.9
total organic carbon (%)	2.6	4.1	12.8	NA[b]	0.6	1.8	2.9
particle size (GMS ± GSD)	23 ± 4	42 ± 44	23 ± 4	NA	38 ± 3	23 ± 5	21 ± 5
mineralogic analysis	**% Pb Mass Distribution in Mineral Phases**						
phase							
anglesite	29	53	11	17	62	50	40
galena	19	24	4		6	6	1
manganese–lead oxide	13		47	3		1	8
lead phosphate	26	8	10	30		1	3
iron-lead oxide		4	11	10			4
iron-lead sulfate	8	7	4	6	32	21	3
cerussite	5			9		21	28
slag			5				
metal-lead oxide[a]			5	15			4
lead oxide		3		4			9
lead-organic carbon			1				
elemental lead			2	6			
no. of particles counted	302	160	94	142	136	303	174

[a] Metals in metal–lead oxide are primarily Zn, Cu, As, Ba, and Cr. [b] NA = not available.

TABLE 2

Arsenic Test Material Composition

sample origin	Anaconda residential soil no. I	Anaconda residential soil no. II	Anaconda house dust no. I
sample identifier	ARS-I	ARS-II	AHD-I
arsenic concn (mg/kg)	3900	410	170
pH	6.6	7.8	7.6
total organic carbon (%)	7.4	12	42
particle size (GMS ± GSD)	19 ± 23	25 ± 4	31 ± 3
mineralogic analysis	**% As mass distribution in mineral phases**		
metal–arsenic oxide[a]	51	46	58
iron–arsenic oxide	35	17	9
metal–arsenic sulfide[b]	1	7	11
arsenic phosphate	1	7	6
slag	7	7	8
iron–arsenic sulfate	3	5	1
metal–arsenic silicate[c]	2	11	7
no. of particles counted	306	587	207

[a] Metals in metal–arsenic oxide are primarily Cu, Zn, Fe, and Al in varying proportions. [b] Metal–arsenic sulfides are a combination of enargite (Cu_3AsS_4), arsenopyrite (FeAsS), and complex solid solutions containing Cu, Te, Pb, Bi, or other metals. [c] Metals in metal–arsenic silicate are primarily Fe and Al in varying proportions.

one composite house dust sample (AHD-I) from the vicinity of a historical copper smelter in Anaconda, MT, while As bioavailability from these three samples was determined in either New Zealand White rabbits or *Cynomolgus* monkeys (*17*, *15*, respectively).

Methods

Selection of PBET Parameters. Rationale for the selection of PBET parameters for gastric and small intestinal pH values, soil mass, and fluid volume, stomach mixing and emptying rate, and small intestinal transit time is described below. The rationale for selection of stomach and small intestinal fluid composition, titration of reaction fluid pH on entering the small intestinal phase, and the method for collection of *in vitro* extract samples are discussed in a previous publication (*10*). In cases where only limited information was available to support selection of a test parameter, a conservative value was selected to maintain the overall conservative nature of the test.

Gastric and Small Intestinal pH. Because pediatric gastric pH is quite variable among individuals, and depends strongly on nutritional status, selecting an appropriate value is a difficult task. Research on pediatric gastric pH using both *in vivo* (ref 23, pH electrode emplaced in lower esophagus, n = 154) and *in vitro* (ref 24, aspiration of stomach fluid followed by pH measurement, n = 105) measurements of pH resulted in mean fasting pH values of 1.7–1.8. Both studies recorded fasting pH ranges from 1 to 4. Following ingestion of food, pediatric gastric pH values rise to >4 (*23*) and subsequently return to basal values within 2 h as food is emptied from the stomach. This behavior is consistent with adults, whose mean fasting gastric pH of approximately 2.0 (*25*) increases to 4–5 following ingestion of a meal (*26*). Gastric pH values

pediatric small intestinal transit time of 3.5 h. It should be noted that orocoecal transit times following ingestion of a fluid meal are considerably shorter: approximately 60 min (*32*, *33*). On the basis of these data, a 4-h small intestinal transit time was selected for the PBET.

Sample Preparation. All samples were oven dried (24 h at 50 °C) and sieved to <250 μm. Lead and As concentrations in the test materials were measured by digestion [method 3050 (*34*)] and GFAA [methods 7420 and 7060, respectively (*34*)]. Sample pH was measured using the soil slurry method [method 9045 (*34*)]. Total organic carbon (TOC) was measured by weight loss on ignition at 430 °C. Particle size distribution was measured using the electrozone method (Particle Data Laboratories, Elmhurst, IL). Lead and As mineralogies were evaluated using an electron microprobe by the method of ref 2 to identify Pb- and As-bearing phases. Lead and As mass distribution among phases in each sample was calculated by correcting the percent occurrence data by the Pb or As concentration and specific gravity of each phase and normalizing the resultant data to 100%.

PBET Procedure. Gastric solution for the PBET was prepared by adjusting 1 L of DI water to the selected pH with 12 N HCl and adding 1.25 g of pepsin (activity of 800–2500 units/mg), 0.50 g of citrate (Fisher Chemical Co.), 0.50 g of malate (Aldrich Chemical Co.), 420 μL of lactic acid (synthetic syrup), and 500 μL of acetic acid (Fisher Chemical Co.). All chemicals were from Sigma Chemical Co. unless otherwise noted. Forty mL of stomach solution was combined with 0.4 g of test material in a 250-mL polyethylene separatory funnel. The funnel was submerged approximately half-way in a temperature-controlled water bath maintained at 37 °C (Figure 1). The substrate/stomach solution mixture was allowed to stand (no agitation) for 10 min, after which argon gas was purged through the reaction vessel at 1 L/min to provide mixing, as described in the section on Stomach Mixing. The pH was checked after 5 min, and every 10 min thereafter, and the pH was adjusted with HCl as necessary. Samples (2 mL each) were collected at 20, 40, and 60 min and centrifuged at approximately 2100*g* for 25 min, and the liquid fraction was decanted. The 2-mL sample volume was replaced with gastric solution to maintain a 40-mL volume in the reaction flask. After 1 h, the reaction was titrated to pH 7 by adding a 5-in.-long dialysis bag (8000 MWCO, Spectra/Por cellulose ester tubing) containing approximately 1 g of $NaHCO_3$ and 2 mL of DI water. The exact amount of $NaHCO_3$ was determined by calculating the amount of HCl added to each vessel and the amount of $NaHCO_3$ necessary to neutralize it. The dialysis bag was removed when the reaction vessel reached pH 7, and 70 mg of bile salts (porcine) and 20 mg of pancreatin (porcine) were added. Samples (2 mL) were obtained from the small intestinal incubation at 1 and 3 h after the reaction flask reached equilibrium at pH 7. Lead and As concentrations in the extracts were determined by ICP [method 6010 (*34*)].

The bioaccessibility of Pb from sample BMW-I was determined using the PBET in triplicate at gastric pH values of 1.3, 2.5, and 4.0. Sample BVS was evaluated at gastric pH values of 1.3 and 3.0, and all of the other Pb substrates were analyzed at gastric pH values of 1.3 and 2.5. Both samples ARS-I and ARS-II were evaluated at gastric pH values of 1.3 and 2.5, and sample AHD-I was evaluated at a pH of 2.5. Soluble Pb and As spikes (spiked at 5 mg/L) were performed at pH values of 1.3 and 2.5 to evaluate recovery of soluble Pb and As (no soil present) from the test system. Pb and As bioaccessibility (%) was calculated as the fraction present in the fluid phase divided by total Pb or As in the reaction vessel, times 100.

Results

Lead concentrations in the seven substrates tested ranged from 1388 to 10 230 mg/kg (Table 1), providing an order-of-magnitude range in the Pb concentrations tested. Arsenic concentrations ranged from 170 to 3900 mg/kg (Table 2). Sample pH values reflected sample provenance, with tailings and mine-waste materials producing acidic pH values (range of 2.4–4.9) and residential soils and house dust producing near-neutral pH values (range of 6.6–7.8). TOC varied, as expected, based on the origin of the test materials. The tailings-derived materials exhibited TOC values of 0.6–2.9%, the Butte mine-waste materials contained 2.6–4.1% TOC, and the residential soils from Bartlesville and Anaconda ranged from 7.4 to 12.8% TOC (Tables 1 and 2). The Anaconda house dust contained 42% TOC, consistent with house dusts containing a sizable component of exfoliated skin cells, hair, food particles, mites, and insect parts (*35*). Volume-based particle size distribution was determined to provide the geometric mean size (GMS) and geometric standard deviation (GSD) of the test substances. The GMS ranged from 19 to 42 μm for the test substances, indicating that particles were within the size fraction (<100 μm) that adheres to children's hands and may be ingested (*36*).

Pb and As Mineralogy. Results from the electron microprobe analyses (Table 1) indicate that the Pb mass distribution in the mineral phases of samples BMW-I and BMW-II, from Butte, are dominated by galena (PbS), its oxidation product anglesite ($PbSO_4$), and lead phosphates of variable composition. The occurrence of anglesite and galena is consistent with a mining waste provenance, as is the observation that Pb particles in BMW samples were frequently encapsulated (or included) in sulfide (pyrite) or silicate (quartz, feldspar) minerals. In contrast, sample BVS, from Bartlesville, was dominated by soil alteration phases (manganese–lead oxide, iron–lead oxide, and lead phosphate); Pb phases resulting from natural soil weathering processes. This observation is consistent with the average of 66% of mineral-phase Pb attributed to soil Pb alteration phases in Bartlesville soils (*21*). In addition, Pb particles in Bartlesville soils were generally not included within other mineral phases (i.e., they were present as liberated particles), rendering them available for dissolution. Similarly, sample SCS contained primarily soil alteration Pb phases, with the addition of metal–lead oxides (metal = As, Zn, and Cu) and cerussite (Table 1) to the Pb phases occurring in sample BVS. The tailings material samples (CT-1 and CT-2) exhibited a lead mineralogy consistent with a surficial tailings environment, comprising galena, anglesite, and an iron–lead sulfate with a chemistry representative of lead jarosite [$PbFe_6(SO_4)_4(OH)_{12}$]. Cerussite ($PbCO_3$), which was observed in one of the samples, was a primary mineral phase in the orebody from which the tailings were derived. Lead solubility from the tailings materials will be controlled by the limited solubility of anglesite in gastric fluid (3) and by the rinding of anglesite and cerussite particles by Pb jarosite, which is stable in acidic solutions [pH < 4, (*37*)]. The sample composed of tailings mixed with stream channel material (CT-3) contained a Pb mineralogy similar to the two tailings samples (CT-1 and CT-2), with the addition of

TABLE 3

Comparison of Pb PBET Results (% Bioaccessibility) and Pb Bioavailability Data

time[a] (h)	BMW-I			BMW-II		BVS		SCS	
	pH 1.3[b]	pH 2.5[b]	pH 4.0[b]	pH 1.3[d]	pH 2.5[d]	pH 1.3[c]	pH 3.0[d]	pH 1.3[c]	pH 2.5[b]
0.33 stomach	7.0 ± 2.0	2.7 ± 1.6	1.1 ± 0.6	22	6	68	22	72	11 ± 4
0.66 phase	8.3 ± 2.0	3.1 ± 2.1	1.1 ± 0.8	31	10	70	25	75	20 ± 2
1.0	9.5 ± 0.4	3.8 ± 1.6	1.3 ± 0.6	35	13	69	26	83	22 ± 6
2.2 small	3.6 ± 2.7	0.94 ± 1.2	0.84 ± 0.2						
3.2 intestine	2.0 ± 0.4	1.4 ± 0.6	0.60 ± 0.2	4	5	16	13	27	8 ± 2
4.2 phase	1.0 ± 1.4	0.94 ± 0.2	0.48 ± 0.2	4	3	12	11	25	7 ± 0
rel Pb bioaccessibility (%) based on stomach data[e]	9.5	3.8	1.3	35	13	70	26	83	22
rel Pb bioaccessibility (%) based on small intestinal data[f]	4.6	2.7		8.3	9.8	29	29	54	18
relative Pb bioavailability in rats[g] (%)		9.3		22.5		35		41	

time[a] (h)	CT-1		CT-2		CT-3		soluble Pb spike	
	pH 1.3[d]	pH 2.5[d]	pH 1.3[d]	pH 2.5[d]	pH 1.3[d]	pH 2.5[d]	pH 1.3[c]	pH 2.5[c]
0.33 stomach	12	6.8	8	4	39	22	95	96
0.66 phase	14	8	9	6	42	24	99	98
1.0	16	8	10	6	49	24	108	104
2.2 small	1.2	0.3	0.7	0.7	5	7	53	41
3.2 intestine	1.7	0.2	0.4	1.0	8	7	43	41
4.2 phase								
rel Pb bioaccessibility (%) based on stomach data[e]	16	8	10	6	49	24	48	41
relative Pb bioaccessibility based on small intestinal data[f]	3.0	0.6	1.1	2.1	14	17		
rel Pb bioavailability in rats[g](%)	14.7		8.7		36			

[a] Time from start of PBET assay. [b] Average ±95% upper confidence limit (1.96σ) on triplicate PBET results. [c] Average of duplicate PBET results. [d] Single PBET test. [e] Relative Pb bioaccessibility values based on stomach data are maximum solubilized Pb in the stomach phase, corrected for maximum recovery of soluble Pb spike in the stomach phase. [f] Relative Pb bioaccessibility (%) based on small intestinal data are average solubilized Pb in the small intestinal phase, corrected for the average recovery of soluble Pb spike in the small intestinal phase. [g] Values presented are relative Pb bioavailability in rats, based on blood-Pb data from dosed-feed studies in Sprague-Dawley rats.

soil Pb alteration phases (e.g., ferromanganese lead oxides, lead phosphate, and metal–lead silicate, Table 1). The presence of more soluble Pb phases (e.g., lead oxide, cerussite, and lead silicates) in the stream channel sample suggests that it will yield a greater fraction of bioaccessible Pb than the two tailings material samples. It should be noted that surface-adsorbed Pb (not detectable by electron microprobe) also constitutes a fraction of the bulk Pb pool that will probably be readily desorbed and more bioaccessible in the acidic gastric environment than the mineralogic phases.

Arsenic mass distribution was similar in samples ARS-I, ARS-II, and AHD-1 and consisted primarily of metal–As oxides (metal = Cu, Zn, Fe, and Al in varying proportions) and iron–arsenic oxides, with minor As contribution from slag, metal–arsenic silicate, As phosphate, metal–arsenic sulfides, and iron–arsenic sulfate (Table 2). As with Pb, surface-adsorbed As most likely constitutes a fraction of the bulk As pool in soil, and is generally adsorbed to hydrous iron and aluminum oxides.

PBET Results for Pb. Triplicate PBET analyses of sample BMW-I at three pH values indicated reproducible results, with reproducibility generally better in the gastric than the small intestinal portion of the test, based on upper 95% confidence limits on the triplicate analyses (Table 3). Results indicate that Pb dissolution in the gastric environment is strongly pH dependent, with an average 57% decrease in soluble Pb when gastric pH was raised from 1.3 to 2.5 (3.0 for BVS) for the seven samples tested. Increasing stomach pH from 2.5 to 4.0 for BMW-I resulted in a further 66% decrease in stomach-solubilized Pb for sample BMW-I (Table 3). On entering the small intestinal phase, solubilized Pb decreased by 74 ± 18%, consistent with extensive adsorption and precipitation reactions removing Pb from solution as the pH increased. It should be noted that solubilized Pb in the test is operationally defined as that portion of Pb remaining in solution after relatively low-speed centrifugation (2500g for 25 min). Solubilized Pb in the small intestinal phase remained generally constant or decreased slightly as the incubation time increased for all seven test substrates. Soluble Pb spikes were approximately 100% recovered in the stomach phase of the test, independent of pH, with recoveries of approximately 48 and 41% in the small intestinal phase when gastric pH values were 1.3 and 2.5, respectively (Table 2).

Comparison of the PBET Pb bioaccessibility values for the seven substrates indicates that the model is sensitive to changes in substrates, with tailings and mine waste producing less bioaccessible Pb than the Pb-bearing soils. These results are consistent with Pb solubility expectations for the various substrates based on the Pb mineralogy data. Galena, anglesite, and lead jarosite contained less bioaccessible Pb than did Pb phases such as lead oxide, cerussite, manganese–lead oxide, and metal–lead oxide. Lead bioaccessibility also varied with pH and TOC of the test material. Acidic pH of the test material results in decreased Pb bioaccessibility, most likely due to formation of soil alteration Pb phases such as anglesite and lead jarosite that are stable in the acidic gastric environment. Neutral soil pH results in soil alteration phases such as cerussite

TABLE 4

Comparison of As PBET Results (% Bioaccessibility) and As Bioavailability Data

	ARS-I		ARS-II		AHD-I	soluble As spike	
time[a] (h)	pH 1.3[b]	pH 2.5[c]	pH 1.3[b]	pH 2.5[c]	pH 2.5[c]	pH 1.3[c]	pH 2.5[d]
0.33 stomach	45	33	43	38			83 ± 4
0.66 phase	49	41	48	44	29	102	93 ± 11
1.0	55	40	49	45	34	103	90 ± 16
2.2 small	50	34	44	30	36	96	99 ± 20
3.2 intestinal phase	50	30	44	32	32	98	95 ± 17
rel As bioaccessibility[e] (%)	50	32	44	31	34		
rel As bioavailability (%) in animal models[f]	48 (rabbits)		20 (monkeys)		28 (monkeys)		

[a] Time from start of PBET assay. [b] Single PBET tests. [c] Averages of duplicate PBET results. [d] Average ±95% upper confidence limit (1.96σ) on five PBET runs. [e] Relative As bioaccessibility (%) calculated as average soluble As mass in small intestinal simulation divided by total As mass in the reaction vessel, corrected for recovery of soluble As spike in small intestinal simulation. [f] Values presented are As bioavailability from soil (dosed in capsules to fasting animals) based on recovery of urinary arsenic relative to recovery of As from sodium arsenate dosed by gavage (*14, 15*). Bioavailability of sodium arsenate (administered by gavage) was 50 and 68% in rabbits and monkeys, respectively, compared to intravenous sodium arsenate.

and ferromanganese lead oxides that appear to have greater solubility in the gastric environment. In addition, organic carbon may provide a sorption surface for soil Pb that will be readily desorbed in the gastric environment, causing elevated soil TOC to result in a greater fraction of bioaccessible Pb, consistent with the observed results (Tables 1 and 3).

Comparison of PBET Results for Pb to Animal Studies. Dosed-feed Pb bioavailability studies in Sprague-Dawley rats were performed such that Pb-bearing soil blended in feed could be compared to lead acetate (a soluble Pb salt) blended in feed, yielding the Pb bioavailability from soil relative to Pb acetate. In addition, lead acetate blended in feed was compared to an intravenous injection of lead acetate (100% bioavailability) to determine the absolute bioavailability of lead acetate (*38*). All of the Pb bioavailability data reported herein are based on measurements of blood Pb in rats.

As previously discussed, for the purpose of developing a bioavailability parameter for exposure assessment, measures of relative bioavailability (i.e., bioavailability of Pb from the test material relative to the bioavailability of Pb in water) are more useful than absolute bioavailability. Measures of relative bioavailability were developed in the Sprague-Dawley rat model for test substrates BVS (*21*), SCS (*7*), and BMW-II (*6*), whereas measures of absolute Pb bioavailability were developed for the other Pb substrates (*22*). However, absolute Pb bioavailability may be corrected to obtain relative bioavailability values, based on the use of a correction for the 15% bioavailability of lead acetate in a dosed-feed Sprague-Dawley rat bioavailability study (*38*). For example, material BMW produced 1.4% absolute Pb bioavailability (*22*), which when corrected for the bioavailability of lead acetate (1.4/0.15), yields an estimate of 9.3% relative Pb bioavailability. Relative Pb bioavailability estimates for all seven test substrates are presented in Table 3.

The PBET data were treated in a similar manner (bioaccessibility of Pb from the test substrate was corrected for the recovery of a soluble Pb spike) to arrive at relative bioaccessibility values for both the stomach and small intestinal phase data (Table 3). The recovery of the soluble Pb spike in the stomach phase (100% at both pH 1.3 and 2.5) was used to correct the stomach phase bioaccessibility values, and the recoveries of the soluble Pb spike in the small intestinal phase (48 and 41% at pH values of 1.3 and 2.5, respectively) were used to correct the small intestinal phase bioaccessibility.

Relative Pb bioaccessibility (X axis) and relative Pb bioavailability (Y axis) values were compared using a linear regression model. At a simulated stomach pH of 2.5, this model yields an r^2 of 0.93 ($n = 7$), based on bioaccessibility calculated from the stomach phase data, and an r^2 of 0.76, based on the small intestinal phase data. The difficulty in achieving a good correlation based on small intestinal phase data is most likely due to the complex nonequilibrium chemical system for Pb in the small intestinal phase (described in ref 3), causing poor reproducibility of the test system and variability in the fraction of Pb that was precipitated or adsorbed during the small intestinal simulation. Although use of the small intestinal phase data would be preferable as a measure of Pb bioaccessibility, the correlation between *in vitro* and *in vivo* results was considerably greater for the stomach phase data. In addition, the correlation between stomach phase relative Pb bioaccessibility and relative Pb bioavailability values was equally good at stomach pH values of 1.3 and 2.5 ($r^2 = 0.93$ in both cases). Although identical r^2 values at pH levels of 1.3 and 2.5 may be coincidental (with only seven observations, it is not possible to establish the significance of this finding), these data suggest that extractability of Pb varies consistently as a function of pH over the range from 1.3 to 2.5 for the seven materials tested.

For the purpose of estimating relative Pb bioavailability in Sprague-Dawley rats based on PBET data, a simulated stomach pH of 2.5 was selected as the most appropriate value, because the linear regression for the pH 2.5 data yielded a y intercept closer to 0 (3.2 for pH 2.5 versus 6.8 for pH 1.3) and a slope closer to 1 (1.4 for pH 2.5 and 0.44 for pH 1.3). These data suggest that the stomach pH of 2.5 more closely resembles the conditions found in the Sprague-Dawley rat during a dosed-feed Pb bioavailability study than the pH value of 1.3.

PBET Results for Arsenic. In contrast to Pb, As dissolution did not exhibit a strong pH dependency, with dissolved As from ARS-I and ARS-II (stomach phase) decreasing by only 25 and 8%, respectively, as system pH was raised from 1.3 to 2.5 (Table 4). These data suggest that stomach pH is less important in controlling As bioaccessibility than it is for Pb. On titration to pH 7, solubilized As decreased by only 20 ± 10%, compared to 60 ± 14% for Pb, consistent with the lack of adsorption and

precipitation reactions involving As at neutral pH conditions (*39*).

Arsenic bioaccessibility (%) derived from the PBET (Table 4) was calculated by averaging the two small intestinal values (duplicate measurements ensured that the small intestinal simulation had reached equilibrium As concentration). These values represent the fraction of As from the test material that is dissolved in the small intestinal fluid and is therefore bioaccessible. Subsequently, dissolved As from the test material was divided by the fraction of soluble As recovered from the soluble As spike, yielding As bioaccessibility relative to a soluble As source (i.e., As in drinking water). Because As recovery from the soluble As spike was close to 100% for both pH 1.3 and 2.5 (Table 4), the spike recovery was assumed to be 100% for the purpose of correcting the PBET data. Relative arsenic bioaccessibility estimated by this method for ARS-I was 50 and 32% at pH values of 1.3 and 2.5, respectively, and 44 and 31%, respectively, for ARS-II (Table 4). House dust (AHD-I) relative arsenic bioaccessibility estimated by this method was 34% (stomach pH of 2.5). These data confirm the difference in As solubility between residential soils and house dusts and soluble As forms, with the soil and house dust As being approximately 3 times less bioaccessible than soluble As at a stomach pH of 2.5.

Comparison of PBET Results for As to Animal Studies. Because fasting stomach pH in rabbits is approximately 1.3 (*10*), it is most appropriate to use pH 1.3 PBET data for comparison to an As bioavailability study in fasted rabbits (soil ARS-I). For the purpose of establishing relative As bioavailability from ARS-I, the absolute As bioavailability in rabbits (24%) was corrected for the bioavailability of soluble As in rabbits (50%), yielding a relative bioavailability of 48% (*17*). Application of a similar calculation to the PBET data for ARS-I at a stomach pH of 1.3 yields a relative As bioaccessibility of 57%, comparable to the rabbit data (48%).

Relative As bioavailability was evaluated for materials ARS-II and AHD-I in *Cynomolgus* monkeys, because this animal model has greater physiological and anatomical similarity to humans than does the rabbit. For the purpose of establishing relative As bioavailability from ARS-II, the absolute As bioavailability in fasted monkeys (14%) was corrected for absorption of soluble As (68%), yielding a relative As bioavailability of 20% (*38*). A similar calculation for house dust, which produced 19% absolute bioavailability, yielded a relative As bioavailability of 28% (Table 4). By a similar calculation, the PBET relative As bioaccessibilities from ARS-II were 44 and 31% at pH values of 1.3 and 2.5, respectively, and 34% from AHD-I at a pH of 2.5 (Table 4). No stomach pH data were found in the literature for *Cynomolgus* monkeys. Monkeys of the species *Macaca mulatta*, the most closely related species to *Cynomolgus* monkeys (*Macaca fascicularis*), have a fasting stomach pH of 1.8 to 2.0.. Therefore, data from the pH 2.5 PBET were likely to be most comparable to the As bioavailability study results in monkeys. Indeed, comparison of these data indicate that the PBET results using a stomach pH of 2.5 provide a conservative estimate of relative As bioavailability for ARS-II and AHD-I in monkeys (the PBET overestimates monkey results by 4−11%).

Discussion

This study demonstrates that the PBET is useful in evaluating the geochemical and physiological factors controlling the bioaccessibility of Pb and As in the gastrointestinal tract. Lead bioaccessibility was observed to be more dependent on stomach pH than was As. When the acidic stomach environment is neutralized, Pb is largely removed from solution by precipitation and adsorption reactions, while As is not.

PBET data were consistent with lead speciation results. Sample BMW-I and BMW-II, which contained primarily less soluble Pb phases (e.g., galena, anglesite, and lead phosphate; Table 1) and a greater degree of Pb phase encapsulation, resulted in minimal Pb bioaccessibility. The Pb-bearing soil samples (BVS and SCS), which contained more soluble Pb phases (metal−lead oxide, lead oxide, cerussite), produced greater fractions of bioaccessible Pb. In addition, the samples derived from tailings materials (CT-1 and CT-2) also produced limited Pb bioaccessibility, due to the presence of anglesite and lead jarosite, which have limited solubility in the acidic gastric environment. Arsenic speciation results indicated that the soil samples ARS-I and ARS-II contained similar As mineralogy, consistent with the nearly identical As bioaccessibility observed for these samples during PBET evaluation. Because mineralogy and bioaccessibility results for ARS-I and ARS-II are nearly identical, differences between rabbit and monkey study results are most likely due to differences between the animal models, rather than between the substrates tested.

The PBET model provided consistent results across a wide pH range and was sensitive to different types of Pb-bearing materials, as reflected in the ability of the test to accurately predict different Pb bioavailability values measured in a Sprague-Dawley rat model. Application of the PBET data at a stomach pH of 2.5 in a linear regression model (relative Pb bioaccessibility on *Y* axis) yielded results that predicted relative Pb bioavailability in rats ($r^2 = 0.93$, $n = 7$). On the basis of this linear correlation, with a y intercept of 3.2 and slope of 1.4, the PBET data can be used to estimate relative Pb bioavailability in the Sprague-Dawley rat model.

Relative Pb bioavailability from the Sprague-Dawley rat model can be used to estimate absolute Pb bioavailability in children, based on the premise that Pb bioavailability from the test substance relative to soluble Pb is similar in both the rat and child models. Interpretation of the data in this manner does not require the assumption that the rat is equivalent to the child, but only that the two Pb forms behave proportionally in the two models. Because only 50% of Pb in the diet (assumed to be soluble Pb) is absorbed by children (*1*), the relative Pb bioavailability from the rat model can be applied to the child by applying a correction factor (0.50) to the relative Pb bioavailability estimate, to arrive at an absolute Pb bioavailability. The absolute Pb bioavailability estimate generated in this fashion can be compared to the EPA default assumption of 30% absolute Pb bioavailability from soil to determine whether a Pb bioavailability correction for soil is warranted.

Results of the PBET indicate good predictive ability for As bioavailability from soil, producing data that are consistent with results from rabbit and monkey As bioavailability studies. Application of the PBET at a stomach pH of 2.5, followed by correction for As bioaccessibility from a soluble As form, results in relative As bioaccessibility data that provide a conservative predictor of relative As bioavailability in monkeys (4−11% overprediction), the animal model likely to best predict As bioavailability in humans. Because the PBET overpredicts As bioavailability

in animal models, a bioavailability study using monkeys is recommended for situations where the accuracy of As bioavailability values is a primary consideration. However, the PBET has been used to develop As bioavailability adjustments ranging from 10 to 25% for soils and house dusts in Oklahoma and Michigan, and these results have been accepted by state regulatory agencies for the purpose of site-specific exposure assessment.

Acknowledgments

The authors wish to thank ARCO, ASARCO, and Cyprus Amax Minerals Co. for funding this research. We are also grateful to Rick Nelson for editing, to Barbara Kyvik for secretarial assistance, to John Drexler and RJ Lee Group for mineralogical analyses, and to three anonymous reviewers for their comments and suggestions. M.V.R. and A.D. are indebted to Paula Diehl and Kathy Davis, respectively, for support over the duration of this research.

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Received for review January 30, 1995. Revised manuscript received September 11, 1995. Accepted September 12, 1995.[⊗]

ES950057Z

[⊗] Abstract published in *Advance ACS Abstracts*, December 1, 1995.